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From: Rao, Manjunath N.  
Sent: Monday, May 19, 2003 8:46 AM  
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Subject: Sequence search request for 10/040863

From: Manjunath N. Rao  
Art Unit 1652, Room 10A11  
Mail Box in Room 10D 01  
Phone: 306-5681

Date: 5-19-03

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Please search the following as soon as possible for application with serial number **10/040863**

1. SEQ ID NO: 7, 9 against all commercial nucleic acid databases including issued patents database and pending application database and provide a print of all results.
2. SEQ ID NO: 8, and amino acids 28-380 of SEQ ID NO:8,
3. SEQ ID NO:10, 11 against all commercial protein databases including issued patents database and pending application database and provide a print of all results.

If you have any questions please call me at the above phone number.

Thanks

Manjunath N. Rao, Ph.D.  
Biotechnology Patent Examiner  
Art Unit 1652, Room 10A11  
Mail Box in 10D01  
Crystal Mall 1, USPTO.

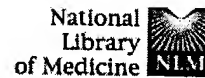
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☐ 1: Eur J Biochem. 1997 Jun 15;246(3):750-5.

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## Structure and expression of the gene encoding secretor-type galactoside 2-alpha-L-fucosyltransferase (FUT2).

Koda Y, Soejima M, Wang B, Kimura H.

Department of Forensic Medicine, Kurume University School of Medicine, Japan.

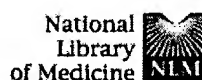
The expression and secretion of ABO antigens in epithelial cells of glands are controlled by secretor-type alpha (1,2)fucosyltransferase activity. We have examined the expression of the secretor-type alpha(1,2)fucosyltransferase gene (FUT2) and a pseudogene of FUT2 (Sec1) in several tumor cell lines by northern blot and/or reverse-transcription-PCR (RT-PCR) analyses. Transcripts of FUT2 were found in total RNA from ovarian, gastric and colonic cancer cell lines but not from six leukemic cell lines, including erythroleukemic HEL cells, by RT-PCR. On the other hand, RT-PCR indicated that Sec1 was expressed in all these tumor cells, including all hematopoietic cells studied. Northern blot analysis indicated that FUT2 transcripts with a similar size (3.3 kb) were expressed in cancer cell lines. Rapid amplification of cDNA ends suggested that the entire FUT2 cDNA is 3.1-kb long and has two Alu repetitive elements in its 3' untranslated region, including an inverted repeat. The mRNA, therefore, may form a large stem-and-loop structure (1.2 kb). Each stem contains about 300 bases, the loop contains 640 bases, and the percentage of complementary nucleotide sequences in the stem region is 85%. The presence of a large stem-and-loop structure in the 3' untranslated region may regulate the level of the FUT2 transcript by affecting the stability of the mRNA.

PMID: 9219535 [PubMed - indexed for MEDLINE]

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☐ 1: J Biochem (Tokyo). 1995 Sep;118(3):541-5.

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## **Purification and characterization of secretory-type GDP-L-fucose: beta-D-galactoside 2-alpha-L-fucosyltransferase from human gastric mucosa.**

**Masutani H, Kimura H.**

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Department of Legal Medicine, Kurume University School of Medicine, Fukuoka.

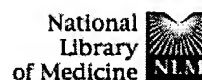
alpha-(1,2)-Fucosyltransferase (GDP-L-fucose:beta-D-galactoside 2-alpha-L-fucosyltransferase) from human gastric mucosa was purified to homogeneity by column chromatographies on Ultrogel AcA34, phenyl-Sepharose, hydroxylapatite, SP-Sephadex, and GDP-hexanol-amine Sepharose. The molecular weight of the purified enzyme was estimated to be 65,000 by SDS-PAGE. The Km value of this enzyme for a type 1 sugar acceptor was a little smaller than that for a type 2 one, indicating this enzyme is a secretor-type alpha-(1,2)-fucosyltransferase. However, the difference between the Km value for a type 1 precursor and that for a type 2 one was very small, suggesting that this enzyme can use both types of precursors as sugar acceptors approximately equally, unlike the purified alpha-(1,2)-fucosyltransferase from human serum as the secretor-type reported previously. The characteristics of the purified enzyme were compared with those of H-type alpha-(1,2)-fucosyltransferase from human plasma. The activities of both enzymes were inhibited by salt and N-ethylmaleimide, but they showed a significant difference in their divalent cation requirements.

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PMID: 8690714 [PubMed - indexed for MEDLINE]

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☐ 1: J Biochem (Tokyo). 1987 May;101(5):1095-105.

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## **The chemical carcinogen-induced enzyme, GDP-fucose: GM1 alpha 1----2 fucosyltransferase in rat liver and hepatoma: modulation by and association with phospholipids.**

**Holmes EH, Hakomori S.**

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Pacific Northwest Research Foundation, Seattle, WA 98104.

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The enzyme GDPFuc:GM1 alpha 1----2 fucosyltransferase, induced by chemical carcinogens in precancerous rat liver as well as rat hepatoma cells, was found previously to be membrane bound, and was inactivated by various detergents, while the activities of many other transferases are generally enhanced by detergents (Holmes, E.H. & Hakomori, S. (1983) J. Biol. Chem. 258, 3706-3717). The effects of phospholipids and detergents on rat hepatoma H35 cells, the conditions of solubilization and subsequent affinity chromatography of the enzyme, and a possible association of phospholipids with the enzyme have been studied with the following major results: The alpha 1----2 fucosyltransferase activity in Golgi membrane was diminished on treatment of membranes with phospholipase A1 or phospholipase C. The enzyme activity was stimulated 7-fold in the presence of cardiolipin or phosphatidylglycerol (and 3-fold by phosphatidylethanolamine) but not other phospholipids. The stimulatory effect of phosphatidylglycerol was eliminated when a variety of ionic or non-ionic detergents were added to the reaction mixture, with the exception of the cationic detergent G-3634-A, which provided a 10-fold total stimulation in the presence of phosphatidylglycerol. The kinetic analysis indicated that addition of phosphatidylglycerol has a negligible effect on apparent Km values but increases the Vmax of the enzyme 5- to 6-fold. The enzyme activity was solubilized by the dialyzable detergent CHAPSO without inhibition of the enzyme activity, and the solubilized enzyme in the presence of 0.4% CHAPSO is partially purified by chromatography on GDP-hexanolamine-Sepharose. Removal of CHAPSO from the affinity purified enzyme by dialysis resulted in a 66% loss of the original activity, which was restored by addition of phosphatidylglycerol. Chromatography of the affinity-purified enzyme with 3H-labeled phosphatidylglycerol on a Biogel A0.5 column indicated an association of the enzyme with the phospholipid that occurred only in the absence of detergent. These results suggest that phospholipid has a direct effect on the enzyme and that the inhibitory effect of detergents can be ascribable to disturbing interaction between phospholipids and the enzyme. A possible role of specific phospholipids on in vivo transferase activity for